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(54) Title: TESTOSTERONE INHIBITORS AND USE FOR THE PROTECTION OF NEURONS

(57) Abstract

The present invention relates to conferring neuroprotection on a population of cells and treating a neurodegenerative disorder by providing an effective dose of a non-estrogen inhibitor of testosterone metabolism wherein the inhibitor excludes four ring cyclopentanophenanthrene compounds.

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TESTOSTERONE INHIBITORS AND USE FOR THE PROTECTION OF NEURONS

Inventors: James Simpkins, Katherine Gordon, Robert Leonard

Technical Field

The present invention relates to novel methods for conferring neuroprotection on a subject that relies on administering an effective dose of at least one testosterone inhibitor.

Background to the Invention

Neurodegenerative diseases have a major impact on society. For example, approximately 3 to 4 million Americans are afflicted with a chronic neurodegenerative disease known as Alzheimer's disease. Other examples of chronic neurodegenerative diseases include diabetic peripheral neuropathy, multiple sclerosis, amyotrophic lateral sclerosis, Huntingdon's disease and Parkinson's disease. Not all neurodegenerative diseases are chronic. Some acute conditions arise from stroke, schizophrenia, cerebral ischemia resulting from surgery and epilepsy as well as hypoglycemia and trauma resulting in injury of the brain, peripheral nerves or spinal cord. There is a need for improved therapeutic agents and methods for reversing or retarding neuronal damage associated with each of these conditions.

Neurodegenerative diseases and aging are characterized by a wide range of symptoms which vary in severity and range from individual to individual. For example, Alzheimer's disease is characterized by symptoms such as depression, aggression, impairment in short-term memory, impairment in intellectual ability, agitation, irritability and restlessness.

A common feature of neurodegenerative disorders and the process of aging in animals is the progressive cell damage of neurons within the central nervous system (CNS) leading to loss of neuronal activity and cell death. This loss of activity has been correlated with adverse behavioral symptoms including memory loss and cognitive deficits. Therapeutic agents that have been developed to retard loss of neuronal activity either have toxic side effects or are prevented from reaching their target site because of

their inability to cross the blood-brain barrier. The blood-brain barrier is a complex of morphological and enzymatic components that retards the passage of both large and charged small molecules thereby limiting access to cells of the brain. There is a need for novel therapeutic agents that are readily transported across the blood-brain barrier as well as for novel methods of treatment of neurodegenerative disorders that directly target the damaged site and are non-toxic.

Traditional methods of treating neurological symptoms focus on: modifying the electrical impulse itself as it moves between and along neurons; or modifying the release or degradation of neurotransmitters. It is now recognized that neuronal cell density has an important impact on function. In various pathological conditions, loss of cell density has been observed resulting from accelerated neuronal cell death. The pattern of degeneration of neurons typically originates from the nerve terminals and progresses "backward" toward the cell body (retrograde degeneration). In several systems, lesioning of certain brain regions results in compensatory sprouting of axons. This plasticity of neurons is attributed at least in part to the presence of trophic growth factors.

These findings have spurred efforts to identify therapeutic agents that compensate for cell loss by stimulating sprouting of dendrites and axons of remaining cells so as to improve the structural integrity of the damaged region. However, the optimal density of neurons and neuronal extensions is a delicate balance between deficiency and excess, a balance that varies with the environment of the cells. This balance can be disrupted when therapeutic agents act on normal or inappropriate tissue. There is a need therefore to target therapeutic agents at a therapeutic dose specifically to those regions where they are required, or, alternatively, to identify agents that have a natural specificity for the target site only, or that are effective at nontoxic doses.

Neurotrophic factors that promote growth and maintenance of cells of the central nervous system (CNS) and sympathetic and sensory neurons of the peripheral nervous system have been investigated for use as therapeutic agents. In particular, the administration of nerve growth factor (NGF), a protein which is normally transported retrogradely in the intact brain from the hippocampus to the septal cholinergic cell bodies as well as from the cortex to the nucleus basalis, provides trophic support to cholinergic neurons and has been shown in animal models to have utility in reducing the effects of neurodegeneration due to trauma, disease or aging. One of the major

problems confronting the use of NGF as a therapeutic agent is finding an appropriate method of increasing the levels of NGF at the appropriate target site. NGF is a large molecule and as such cannot normally pass across the blood-brain barrier and therefore has very limited access to the cells of the brain. Current methods for administering nerve growth factor across the blood-brain barrier include: polymeric implants, osmotic minipumps, cell therapy using genetically engineered autologous or heterologous cells secreting NGF for implantation into the brain, and methods of increasing the permeability of the blood-brain barrier thereby allowing diffusion of these molecules to cells in the brain. Where exogenous NGF is used, a relatively large amount of relatively costly recombinant protein is required. Non-localized targeting not only decreases the amount of protein available at the target site but also results in stimulation of growth of neurons at inappropriate sites resulting in potential harmful effects for the subject.

An additional approach to treating neurological symptoms has followed the observation that certain amino acids (glutamic acid and aspartic acid) act as excitatory neurotransmitters that bind the N-methyl D-aspartate (NMDA) receptor. Excess release of these amino acids (EAA) causes overstimulation of the neurons in neurodegenerative diseases as well as in conditions of hypoglycemia or trauma, resulting in neuronal loss and behavioral dysfunctions. NMDA is a potent and toxic analogue of glutamate which has been shown in animal studies to mediate much of the neuronal death associated with head trauma, hypoglycemia, anoxia, hypoxia and other conditions, and compromises the flow of blood, oxygen or glucose to the central nervous system.

A number of synthetic compounds that act as antagonists of the receptor have been described and tested in animal models. The possibility that these compounds are toxic in humans remains unresolved. Despite many years of clinical research, these antagonists are not as yet available as therapeutic products for treating patients.

Estrogen compounds have been found to have a neuroprotective effect (Simpkins et al., U.S. Patent 5,554,601 herein incorporated by reference). Furthermore, the class of compounds identified as four ring cyclopentanophenanthrene compounds have been shown to have a neuroprotective effect. (Simpkins et al., U.S. Serial No. 08/685,574, herein incorporated by reference). These observations have been confirmed in a variety of *in vitro* and *in vivo* models for neurodegeneration [C. Behl, et al., Biochem. Biophys. Res. Comm., Vol. 216, (1995), pp. 473-482; J. Bishop, et al., Molecular and Cellular

Note 4 citations for activity.

Neuroscience, Vol. 5, (1994), pp. 303-308; Y. Goodman, et al., J Neurochem, Vol. 66, (1996), pp. 1836-1844; P. S. Green, et al., J Neuroscience, Vol. 17, (1997), pp. 511-515; J. W. Simpkins, et al., Neurobiology of Aging, Vol. 15, (1994), pp. S195-S197; C. A. Singer, et al., Neurosci. Let., Vol. 212, (1996), pp. 13-16].

There is a continued need to identify neuroprotective agents to retard neuron loss that plays a significant role in disease progression in neurodegenerative diseases as well as trauma and aging.

Summary of the Invention

A method is provided for conferring neuroprotection on a population of cells in a subject that includes in a preferred embodiment, the steps of providing an effective dose of a non-estrogen inhibitor of testosterone metabolism in a pharmaceutical formulation, wherein the inhibitor further excludes four ring cyclopentanophenanthrene compounds; and administering the inhibitor to the subject so as to confer neuroprotection. In a preferred embodiment, the subject is a male subject.

In a preferred embodiment, the method further includes an inhibitor of testosterone metabolism that is selected from the group consisting of: a hormone, a steroidal anti-androgen, a non-steroidal anti-androgen, an androgen synthesis inhibitor, a luteinising hormone receptor antagonist, a luteinising hormone receptor agonist and a 5-alpha-reductase inhibitor, the compound being preferably administered by a route selected from oral, intramuscular, transdermal, buccal, intravenous and subcutaneous.

In a further embodiment of the invention, a method of conferring neuroprotection on a population of cells in a subject is provided that includes the steps of providing a mixture containing a plurality of non-estrogen inhibitors of testosterone metabolism at an effective dose and in a pharmaceutical formulation, wherein the inhibitor further excludes four ring cyclopentanophenanthrene compounds; and administering the inhibitor to the subject so as to confer neuroprotection.

In a further embodiment of the invention, a method of treating a neurodegenerative disorder in a subject, includes providing an effective dose of a non-estrogen inhibitor of testosterone metabolism in a pharmaceutical formulation wherein the inhibitor further excludes four ring cyclopentanophenanthrene compounds; and administering the formulation to the subject so as to retard the adverse effects of the

disorder.

Brief Description of the Figures

Figure 1 shows the effects of androgen environment on ischemic lesion size in male rats. Depicted are the percentage of the cross-sectional areas of the brain (mean percent ischemic area±SEM) in various slices taken at increasing distances caudal to the olfactory bulb from gonad intact (Intact), castrated (Castrate) and castrated animals with testosterone replacement (Castrate +T). * indicates $p<0.05$ versus intact rats for individual brain sections.

Figure 2 shows the effects of endocrine manipulation on mean percent ischemic area (mean ±SEM) in male rats. * indicates $p<0.05$ versus Intact. ** indicates $p<0.05$ versus Castrate and Castrate + E2 groups. Data is presented from intact rats, castrated rats, castrated rats treated with testosterone, intact rats treated with estradiol, castrated rats treated with estrogen and castrated rats treated with estradiol and testosterone.

Figure 3 shows the relationship between plasma testosterone concentration and mean percent ischemic area in male rats. Depicted are the mean ± SEM for both plasma testosterone concentration and percent mean ischemic area for the six treatment groups evaluated. The r^2 value for the relationship is 0.922.

Detailed Description of the Invention

Neuron loss is associated with disease progression and therefore methods to retard neuron loss are desirable for disease management. Whereas certain classes of compounds have shown efficacy in retarding neuron loss, it is desirable to identify additional compounds that may prove effective at retarding neurodegenerative disease progression and aging as well as the sequelae of trauma. In aging populations, there is a particular need for methods of protecting neurons from cell death caused by neurodegenerative diseases, aging and trauma.

While estrogens have been found to have neuroprotective properties (Simpkins et al., 1996), little is known about the action of the male counterpart - testosterone with regard to neuroprotection.

Testosterone enhances the release of the vasoconstrictor substance, neuropeptide Y, in response to stress [Z. Sukowska-Grojec, Ann, N. Y. Acad. Sci., Vol. 771, (1995),

pp. 219-33]. Additionally, testosterone has been shown to inhibit synthesis of the vasodilator, protacyclin, in aortic tissue [J. Nakao, et al., Atherosclerosis, Vol. 39, (1981), pp. 203-209], to enhance thromboxane A2-induced constriction of coronary arteries [K. Schor, et al., Euro. J. Clin. Inves., Vol. 24 Supp., (1994), pp. 50-52], and to reduce thromboxane A2 receptors in aorta [K. Matsuda, et al., Amer. J. Physiologic., Vol. 267, (1994), pp. H887-H893]. Testosterone has also been shown to enhance platelet aggregation [W. I. Rosenblum, et al., Thromb. Res., Vol. 45, (1987), pp. 719-728] and arachidonic acid-induced thrombosis [A. D. Uzunova, et al., Prostaglandins, Vol. 13, (1977), pp. 995-1002].

Aroonsakul specifically included testosterone in a mixture for treating symptoms of patients with Alzheimer's disease, suggesting that testosterone might have a beneficial effect as a therapeutic agent. (Aroonsakul (1990) U.S. Patent 4,897, 389). However, others have reported that endogenous testosterone does not diminish or enhance neuron survival in male rats with middle cerebral artery occlusion (MCAO) (Toung et al. Stroke, 1998: 29:1666-1670).

In contrast to the foregoing, it is here demonstrated for the first time that testosterone has an adverse effect on retardation of neuron loss (see Examples). Consequently, this observation provides for the use of testosterone inhibitors to reverse the negative effect of testosterone on neuron loss so as to enhance neuroprotection. Testosterone inhibitors known in the art include compounds for treating prostate cancer. In a preferred embodiment of the invention, a new use is provided for these compounds and other testosterone inhibitors as defined below.

According to the invention, testosterone inhibitors may be used singly or in combination to prevent neuron loss which occurs following an injury or disease. Testosterone inhibitors may be used to treat neuron loss in conditions that include but are not limited to: stroke, transient ischemic events, subarrachinoid hemorrhage, neuron loss secondary to cardiac or neural surgery, shock, head trauma, Alzheimer's disease, Parkinson's disease, Huntingdon's disease, AIDS, dementia, aging and schizophrenia.

"Testosterone inhibitors" are defined here and in the claims as compounds which decrease the concentration or activity of testosterone or inactivate or otherwise antagonize or inhibit the activity or metabolism of testosterone which would otherwise lead to neuron loss. According to the definition, this class of compounds does not

include four ring cyclopentanophenanthrene compounds or estrogen compounds. The testosterone inhibitor molecules include antagonists and agonists of testosterone that counteract the action of testosterone. Without wishing to be limited to scientific theories, testosterone inhibitors used in the invention, may act for example by binding to the androgen receptor, interfering with nuclear accumulation of active receptor-hormone complexes, by down-regulating the synthesis testosterone or acting on the metabolism of testosterone. The testosterone inhibitor molecules used according to the invention may include compounds found to be effective in treating prostrate cancer. Testosterone inhibitors are not limited to but are exemplified by: (1) Hormones which inhibit hypothalamic release of gonadotrophin releasing hormone (GnRH); (2) GnRH analogues (e.g., goserelin Zoladex), leuprorelin (Prostap), buserelin (Suprefact), triptorelin (De-capeptyl), (nafarelin); (3) steroidal anti-androgens (eg., cytoproterone acetate, megestrol acetate); (4) pure anti-androgens (eg., flutamide (Drogenil), nilutamide, bicalutamide (Cadodex); (5) androgen synthesis inhibitors (eg., ketoconazole); (6) 5-alpha-reductase inhibitors, e.g., finasteroid or Proscen and including inhibitors for treating prostrate cancer; (7) androgen receptor antagonists including cytoproterone, flutamide, cymetidine, ranitidine and spironolactone; (8) agonists and antagonists of the luteinizing hormone releasing hormone (LHRH).

"Estrogen compounds" are defined here and in the claims according to U.S. Patent 5,554,601.

"Polycyclic phenolic compounds" are defined here and in the claims by the compounds enumerated in Simpkins et al. U.S. Serial Number 08/685,574, herein incorporated by reference.

A preferred embodiment of the current invention is directed to the observation that the amounts of testosterone in a male animal is correlated with the extent of ischemic damage following a stroke. The correlation of testosterone as a negative risk factor in the outcome of cerebrovascular ischemia demonstrated in the accompanying example, is novel.

The data shows that plasma testosterone concentrations are highly correlated with increased ischemic brain damage from middle cerebral artery occlusion. The data is obtained using a rat model in which middle cerebral artery (MCA) occlusion has been used to produce focal ischemic lesions in the rat. This model is the preferred

experimental model for studying neuron loss in the human brain. Reduction in plasma testosterone in the experimental MCA rat model, either through castration or treatment with estradiol, is associated with approximately a 50% reduction in ischemic lesion size.

The lesions produced by the MCA occlusion in male rats occurred in the frontal and parietal cortex and the basal ganglia, with the maximal extent of the lesion seen at 7 and 9 mm posterior to the olfactory bulb. Reduced ischemic damage was further detected in the rostral and caudal to these areas, where the anterior and posterior cerebral arteries, respectively, also supply the tissue [S. A. Menzies, et al, Neurosurgery, Vol. 31, (1992), pp. 100-106]. The brain samples taken from the expected region of the MCA lesion in control rats demonstrated the expected effects of the MCA occlusion. In sample rats that had been castrated, the castration resulted in a reduction of the size of the ischemic lesion by 59%. Testosterone replacement restored lesion size to the extent expected from the levels of plasma testosterone following replacement therapy. A strong positive relationship between plasma testosterone levels and lesion size was observed with a r^2 value of 0.922. These data suggest that regardless of other endocrine factors, plasma testosterone is a primary determinant of the size of ischemic lesions following MCA occlusion in the male rat. In female rats, ovariectomy enhances and estrogen treatment reduces by about 50% ischemic lesion following MCA occlusion [Simpkins, Serial no. 08/749,703, incorporated by reference]. In the example, 17 β-estradiol exerted a profound protective effect in intact male rats that were associated with a marked reduction in plasma testosterone concentrations. In the presence of testosterone from a Silastic® implant, estradiol was only partially effective in reducing lesion size.

Table 1: Effects of Gonadal Steroid Modification on Mortality and Plasma Testosterone Concentration Following Middle Cerebral Artery Occlusion.

Treatment Group	N	Plasma Testosterone ng/ml mean±sem	Mortality (%)
1. Intact	5	1.57 + 0.41*	46
2. Castrate	8	0.07 + 0.03	15
3. Castrate + T	11	0.67 + 0.07**	12
4. Intact + E2	9	0.05 + 0.01	30
5. Castrate + E2	6	0.04 + 0.01	9
6. Castrate + E2 + T	7	0.59 + 0.06**	13

N = Number of rats in each treatment group.
*$p<0.05$ when compared with all other treatment groups.
**$p<0.05$ when compared with Intact + E2, Castrate and Castrate + E2 treatment groups.
Chi Square Analysis of Mortality data revealed insignificant ($p>0.16$) treatment effect on mortality.

Examples

Animals and Gonadectomy: Male Charles River rats weighing approximately 250 g were purchased from the Wilmington, MA colony and were maintained in an AAALAC accredited vivarium for 1 week prior to gonadectomy. All animal procedures were approved by the University of Florida Animal Care and Use Committee. Bilateral gonadectomy was performed under methoxyflurane (Metophane® Pitman Moore, Crossings, NJ) inhalant anesthesia 7 days prior to MCA occlusion.

Steroid Treatments: 17β-estradiol was packed into 5mm long Silastic® tubes and testosterone was packed into 10mm long silastic tubes that were closed on either end with Silastic Medical Adhesive® (Dow-Corning). Sham (empty) pellets were similarly prepared. All pellets were washed with methanol to remove the steroid adhering to the outside of the tubes. Subsequently, pellets were washed in physiological saline, a procedure that assures first order *in vivo* release of estradiol to achieve physiologically relevant concentrations [M. Singh, et al., Brain Res., Vol. 644, (1994), pp. 305-312]. The pellets were implanted subcutaneously (sc) at the time of castration (1 week prior to the MCA occlusion).

Testosterone Assay: The blood from rats for radioimmunoassay (RIA) of

testosterone was collected in heparinized tubes by intracardiac puncture just before sacrifice. The plasma was separated by centrifugation and stored at 80°C until RIA. Coat-A-Count RIA kit was purchased from Diagnostics Products Corporation, Los Angeles, CA. The tracer had high specific activity with approximately 30 to 40% maximum binding. The antiserum used was highly specific for testosterone with little cross reactivity to other compounds. The Coat-A-Count total testosterone assay had a broad reportable range of 4 to 1600 ng/dl, 50 μl of the same was used in duplicate tubes for RIA. The concentration of the unknown samples was read from the standard calibration curve whose correlation coefficient was 0.9989.

Middle Cerebral Artery Occlusion: At 7 days after gonadectomy and steroid implantation, animals were anesthetized with ketamine (60mg/kg, ip) and zylazine (10mg/kg, ip). During surgery, rectal temperature was maintained between 36.5 and 37.0°C by a heating lamp. During an operating microscope, the left carotid artery was exposed through a midline incision of the neck. The sternohyloid, digastric (posterior belly) and the omohyloid muscles were divided and retracted. Then the greater horn of the hyloid bone was removed for exposure of the distal external carotid artery (ECA). The common carotid artery (CCA) was dissected from the vagus nerve and the ECA and its branches (occipital and superior thyroid arteries) were dissected distally. The internal carotid artery (ICA) was carefully separated from the vagus and glossopharyngeal nerves just below the ECA. Near the base of the skull, the ICA has an extracranial branch, the pterygopalatine artery. Beyond this bifurcation, the ICA enters the cranium medially. After the arteries and their branches were dissected, the distal ECA and its branches, the CCA and the Pterygopalatine arteries were cauterized completely. The ECA and the occipital arteries were cut, then a microvascular clip was placed on the internal carotid artery (ICA) near the base of the skull.

The tip of 2.5 cm long 3-0 monofilment nylon suture was heated to create a globule for easy movement and blockade of the lumen of the vessel. The suture was introduced into the ECA lumen through a puncture and was gently advanced to the distal ICA until it reached the clipped position. The microvascular slip was then removed and the suture was inserted until resistance was felt. The distance between the CCA bifurcation and the resistive point was 1.8 cm. The resistance indicated that the suture had passed the middle cerebral artery origin and reached the proximal segment of the

anterior cerebral artery. The operative procedure was completed with 10 min. with minimal blood loss. After 40 minutes of occlusion time, the suture was withdrawn from the ICA and the distal ICA was immediately cauterized.

Quantitation of Mortality and Ischemic Area: Animals that survived until the scheduled sacrifice time (24 hours after MCA occlusion) were killed by decapitation. Prior to sacrifice, cardiac puncture was used to obtain blood samples for subsequent assessment of plasma testosterone concentration. Brains were removed 24 hours after MCA occlusion and placed in a metallic brain matrix (ASI Instruments, Inc., Warren, MI) for slicing. Coronal sections 2 mm thick were made at 5, 7, 9, 11 and 13 mm posterior to the olfactory bulb. The slices were incubated for 30 minutes in a 2% solution of 2, 3, 5 triphenyltetrazolium chloride (Sigma Chemical Corp., St. Louis, MO) in physiological saline at 37°C. Stained slices were photographed and subsequently imaged using a McIntosh Quatra 800 computer, equipped with an Image 1.47 software program for the assessment of the ischemic area of the lesion. The area of the entire sliced brain was outlined and quantified; and, then, the ischemic area was outlined and quantified. The ratio of ischemic area to total brain area was calculated to give the percent ischemic area for each brain slice. The ischemic area in each brain slice was averaged for each animal and the number was recorded. This was done for each animal in each group to produce the mean ischemic area. These images and calculated area of ischemia were stored for later retrieval and data reduction.

Statistical Evaluation of Data: The significance of differences in lesion size among the 6 treatment groups was determined by ANOVA and the Fischer's test was used for the post hoc analysis. $P<0.5$ was considered significant. The correlation between testosterone and the lesion size was analyzed by regression analysis. The significance of the mortality of the rates prior to sacrifice was analyzed by Chi Square analysis.

The effects of castration and testosterone replacement on ischemic damage following MCA occlusion is shown in Figure 1. Intact rats showed the expected rostral to caudal extent of ischemic damage with peak ischemic lesions observed at 7 and 9 mm caudal to the olfactory bulb. Lesion size was small at more rostral and caudal brain sections. The MCA occlusion lesion occupies the expected brain regions, i.e., the frontal and parietal cortex and basal ganglia, supplied by the MCA [S. A. Menzies, et al,

Neurosurgery, Vol. 31, (1992), pp. 100-106]. Castration of adult male rats reduced ischemic lesion size in each section evaluated (Figure 1) and reduced the overall mean ischemic are from 17±3% in intact rates to 8±2% in castrate rates (Figure 2). Testosterone replacement of castrate rats increased lesion size in all sections evaluated (Figure 1) and increased overall mean ischemic area to 14±2% (Figure 2).

Estrogens are neuroprotective against MCA occlusion-induced ischemic brain damage in female rats (U.S. serial number 08/749,703 incorporated by reference). Here, we evaluated the effects of estrogen treatment in males. Treatment of intact male rats with 17β-estradiol reduced overall mean ischemic area from 17±3% to 8±2% (Figure 2). Treatment of castrated males with estradiol did not change the already reduced size of the MCA occlusion-induced lesion (Figure 2). Simultaneous treatment with both testosterone and estradiol in castrated rats resulted in an ischemic lesion of 12±2%, intermediate between that of castrate +T(14±2%) and castrate +T (14±2%) and castrate +E2(7±2%) (Figure 2).

Castrate + E2 treatment profoundly reduced plasma testosterone concentration from 1.556±0.409 ng/ml in intact male rats to 0.069±0.029 ng/ml and 0.054±0.010 ng/ml in castrate and intact + E2-treated animals, respectively (Table 1). Testosterone replacement in castrate or castrate + E2 rats increased plasma testosterone concentration to 0.668±0.067 ng/ml and 0.590±0.055 ng/ml, respectively (Table 1).

We conducted an analysis of covariance to assess the relationship between plasma testosterone and overall mean percent ischemic area (Figure 3). A r^2 of 0.922 was observed when ischemic area was analyzed on the basis of plasma testosterone concentrations.

Mortality (deaths prior to the scheduled 24 hour sacrifice time) was high in the intact group (46%) and the intact + E2 group (30%), but was low (9-15%) in all castrate groups, regardless of their hormone replacement (Table 1).

Claims

We claim:

1. A method of conferring neuroprotection on a population of cells in a subject, comprising:

(a) providing an effective dose of a non-estrogen testosterone inhibitor in a pharmaceutical formulation, wherein the inhibitor further excludes four ring cyclopentanophenanthrene compounds; and

(b) administering the inhibitor to the subject so as to confer neuroprotection.

2. A method according to claim 1, wherein the testosterone inhibitor is selected from the group consisting of: a hormone, a steroidal anti-androgen, a non-steroidal anti-androgen, an androgen synthesis inhibitor, a luteinising hormone receptor antagonist, a luteinising hormone receptor agonist and a 5-alpha-reductase inhibitor.

3. A method according to claim 1, wherein the subject is a male subject.

4. A method according to claim 1, wherein step (b) further comprises administering the inhibitor by a route selected from oral, intramuscular, transdermal, buccal, intravenous and subcutaneous.

5. A method of conferring neuroprotection on a population of cells in a subject, comprising:

(a) providing a mixture containing a plurality of non-estrogen testosterone inhibitors at an effective dose and in a pharmaceutical formulation, wherein the inhibitor further excludes four ring cyclopentanophenanthrene compounds; and

(b) administering the inhibitor to the subject so as to confer neuroprotection.

6. A method of treating a neurodegenerative disorder in a subject, comprising:

(a) providing an effective dose of a non-estrogen testosterone inhibitor in a

pharmaceutical formulation wherein the inhibitor further excludes four ring cyclopentanophenanthrene compounds; and

(b) administering the formulation to the subject so as to retard the adverse effects of the disorder.

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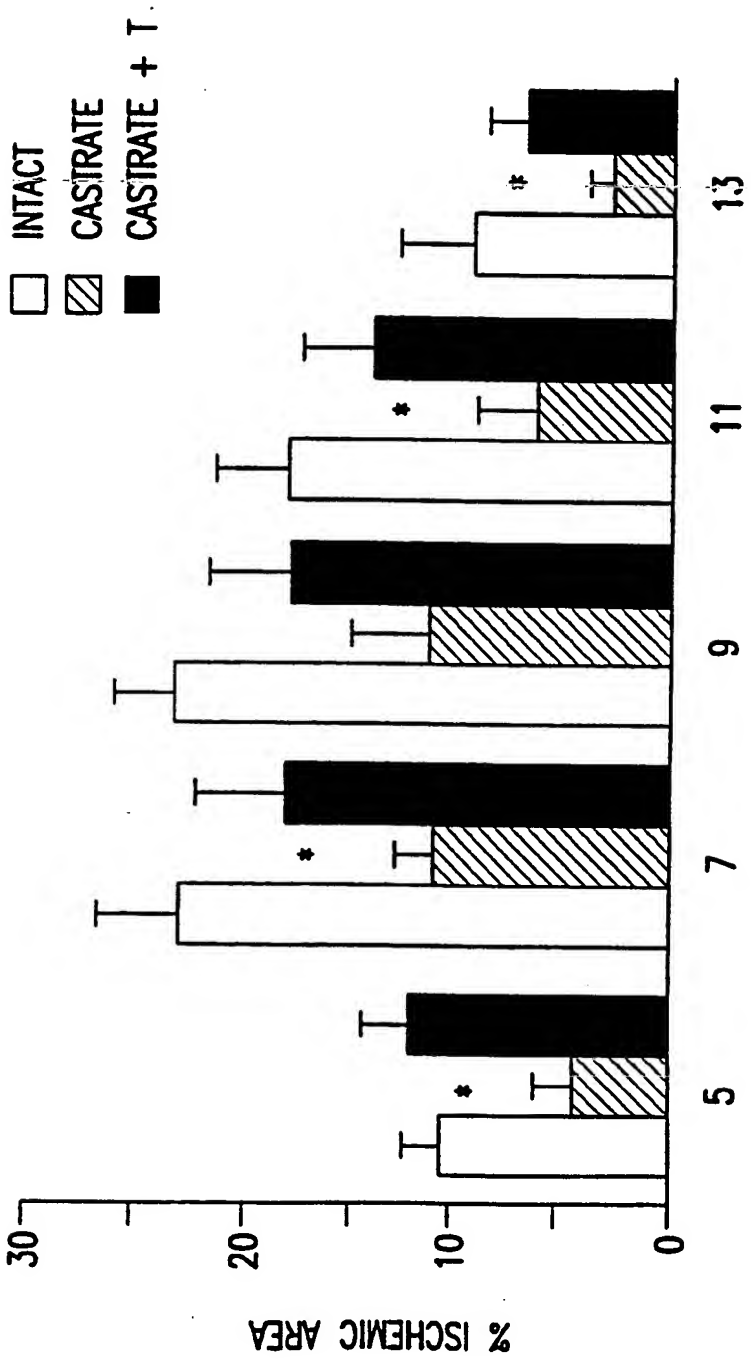
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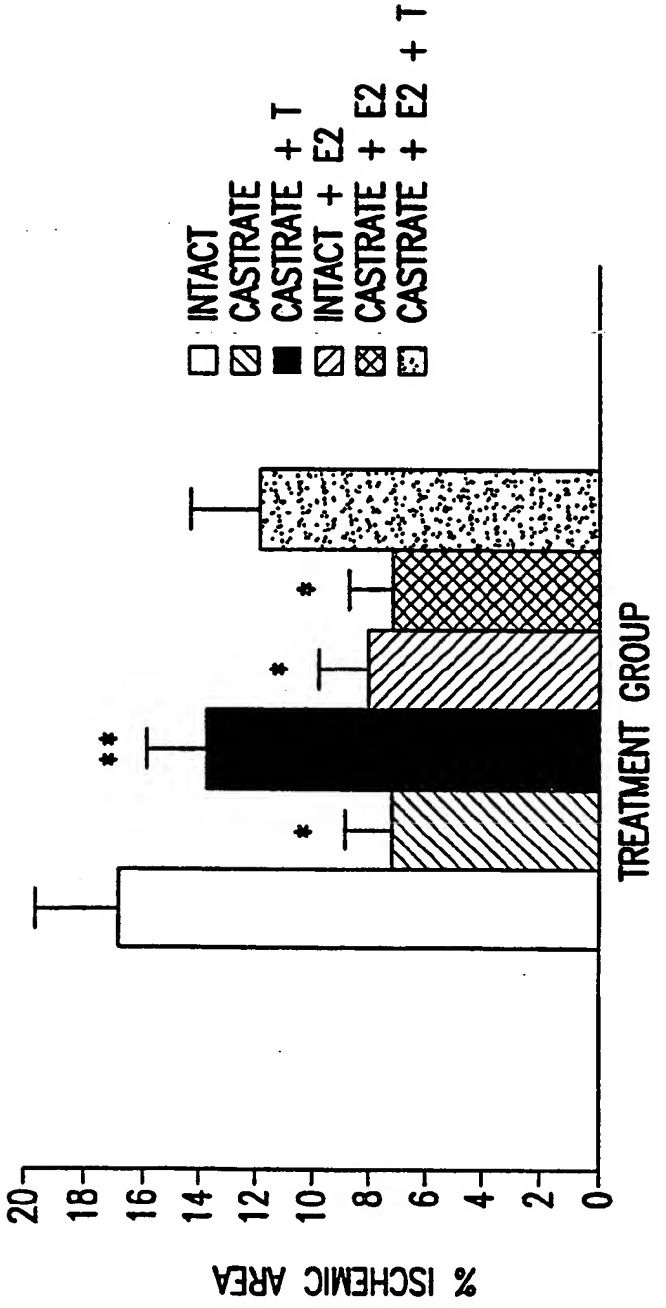
FIG. 1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TREATMENT GROUP

FIG.2

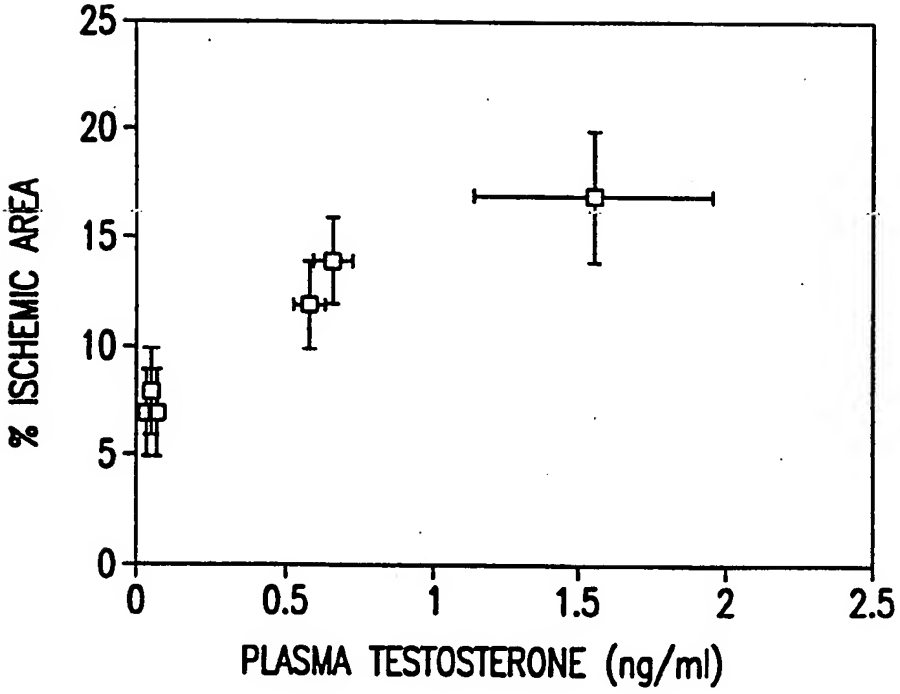
25
20
15
10
5
0
% ISCHEMIC AREA
0
0.5
1
1.5
2
2.5
PLASMA TESTOSTERONE (ng/ml)

FIG.3

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 98/25140

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/56 A61K31/16 A61K31/495 A61K31/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 453 428 A (KAMINSKI RAM) 26 September 1995 see column 2, line 41 - line 51 see claim 7 ---	1-6
X	WO 94 24146 A (HESCH ROLF DIETER) 27 October 1994 see claims 10,11 ---	1-6
X	EP 0 679 642 A (TAKEDA CHEMICAL INDUSTRIES LTD) 2 November 1995 see page 5, line 38 see page 23, line 2 - line 3 ---	1-6
X	DE 43 20 896 A (DENECKE RAINER DR MED VET) 5 January 1995 see the whole document --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 February 1999	15/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Trifilieff-Riolo, S

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 98/25140

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 792 642 A (PFIZER) 3 September 1997 see page 6, line 21 - line 22 see page 19, line 50 - page 20, line 38	1-6
X	RICH ET AL.: "leuprolide acetate for exhibitionism in Huntington's disease" MOV. DISORD., vol. 9, no. 3, 1994, pages 353-357, XP002094942 see abstract	1-6
X	VARTANIAN ET AL: "experience using vespiron in the combined therapy of parkinsonism" ZH NEVROPATOL PSIKHIATR, vol. 88, no. 12, 1988, pages 14-18, XP002094943 see abstract	1-6
X	GEORGIOU ET AL: "reliance on advance information and movement sequencing in huntington's disease" MOV. DISORD., vol. 10, no. 4, 1995, pages 472-481, XP002094944 see page 474, Table I, patient 1	1-6
A	DATABASE WPI Derwent Publications Ltd., London, GB; AN 156568 XP002094950 "agent to prevent brain cell disorder e.g. alzheimer's or senile dementia contg. sex hormone esp. testosterone or oestradiol" & JP 06 100466 A (TSUMURA ET CO) , 12 April 1994 see abstract	1-6

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/25140

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1 to 6
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1 to 6 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
In view of the large number of compounds which are defined by the wording of the claims, the search has been performed on the general idea and compounds specifically mentioned in the description.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 98/25140

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5453428 A	26-09-1995	US 5352688 A	04-10-1994
		US 5177081 A	05-01-1993
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		AU 688739 B	19-03-1998
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		EP 0694038 A	31-01-1996
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		US 5795883 A	18-08-1998
EP 0679642 A	02-11-1995	CA 2148047 A	29-10-1995
		JP 8012650 A	16-01-1996
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